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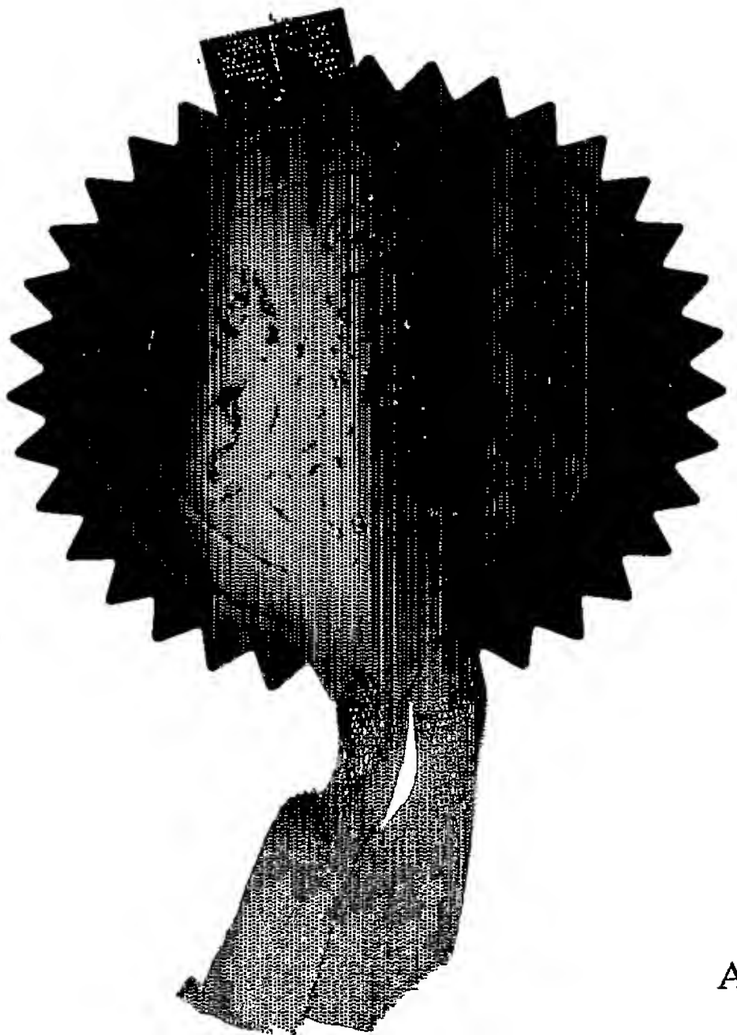

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METHODS

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METHODS

The present invention relates to methods of screening for potential insecticidal agents using genes encoding proteins that regulate xenobiotic detoxification in insects. In particular the invention relates to methods of using insect nuclear hormone receptor genes and/or their promoters, especially the Drosophila *dhr96* gene and/or promoter and homologues thereof, as well as to the products of such insect nuclear hormone receptor genes, to identify and/or optimise potential insecticidal agents. The methods employ transgenic cells and/or whole organisms in which an insect nuclear hormone receptor gene is deleted or under-expressed as well as transgenic cells, cell-lines and/or organisms that over-express an insect nuclear hormone receptor gene or express an insect nuclear horomone receptor gene in a heterologous manner. The invention further extends to the transgenic cells, cell-lines and/or organisms used in the methods of the invention as well as to DNA constructs comprising an insect nuclear hormone receptor gene or a fragment thereof and DNA constructs comprising the promoter of an insect nuclear hormone receptor gene.

Different classes of xenobiotic compounds have been, and are being, used as insecticides. However, resistance to a large number of these compounds has emerged in most insect species. Resistance to xenobiotic compounds may be mediated by one or more different molecular mechanisms e.g. target site alteration (see for example: Mutero *et al.*, 1994, Proc. Natl. Acad. Sci. 91:5922-5926; ffrench-Constant *et al.*,2000, Annu. Rev. Entomol. 45:449-466; Williamson *et al.*, 1996, Molec. Gen. Genet 252:51-60), enzymatic modification or degradation of the xenobiotic (e.g. by P450s, carboxylesterases, glutathione S-transferases; Ranson *et al.*, 2002, Science 298:179-181), and active efflux (Foote *et al.*, 1990, Nature 345:255-258; Lanning *et al.*, 1996, Toxicol Lett. 85:127-133).

Accordingly there exists a need to develop novel insecticides that are capable of by-passing one or more of the above-mentioned molecular mechanisms of resistance.

One conventional method of identifying novel insecticidal compounds relies on screening compounds against wild-type insects and assessing whether the compound has any deleterious effect on the insect. When potential insecticidal compounds are screened for activity against wild-type insects, only very few are shown to be highly insecticidally

active and it is routinely only these compounds that are taken forward into further development as a potential insecticide. The vast majority of compounds, which either exhibit only a low level of insecticidal activity or which have no insecticidal activity at all, are often discarded.

The apparent lack of insecticidal activity of a compound screened against wild-type insects may be due to the fact that the compound is truly incapable of acting as an insecticide. Alternatively, the apparent lack of insecticidal activity may be due to the compound being metabolised by the insect, for example via P450 or other enzyme mediated metabolism, or as a result of active efflux of the compound from insect cells.

It is thus likely that some of the compounds that are routinely discarded, due to their lack of insecticidal activity against wild-type insects, have the potential to be insecticides if they were modified so that they were no longer susceptible to such xenobiotic detoxification mechanisms.

This disadvantage of the conventional method of screening (i.e. the fact that weak chemical leads are often discarded) is addressed by the current invention which uses transgenic insects and insect cells wherein a xenobiotic detoxification pathway has been altered such that xenobiotic detoxification in the transgenic insect or insect cell is reduced relative to xenobiotic detoxification in a wild-type insect or insect cell and these transgenic insects and/or insect cells are thus used to screen for, and identify, weak chemical leads.

The present invention achieves this reduction in xenobiotic detoxification in an indirect manner by reducing the level of expression of one or more key genes that act as a "master-switch" in regulating detoxification (e.g. P450-mediated or other xenobiotic detoxification) pathways in insects. These key genes act upstream of, for example, P450 or *cyp* gene(s) in a P450 metabolic pathway and are thought to act as regulators of *cyp* gene expression. Thus by reducing the level of expression or activity of such a key "master-switch" gene, the level of expression of one or more downstream (e.g. *cyp*) genes is indirectly reduced. The present invention is based on the finding that the nuclear hormone receptor gene *dhr96* from *Drosophila melanogaster* is one such "master-switch".

Thus in a first aspect of the invention there is provided a transgenic insect or insect cell wherein the level of expression of an insect nuclear hormone receptor gene has been

reduced relative to the level of expression of said nuclear hormone receptor gene in a non-transgenic insect or insect cell.

For the avoidance of doubt the term " transgenic" as used herein in reference to an insect or insect cell, describes an insect or an insect cell wherein the genetic material of said insect or insect cell has been altered using recombinant DNA technology. Thus, a transgenic insect or insect cell of the invention does not include a naturally occurring or chemically produced mutant insect or insect cell wherein the level of expression of a nuclear hormone receptor gene has been reduced relative to the level of expression of said nuclear hormone receptor gene in a non-mutant or wild-type insect or insect cell.

The nuclear hormone receptor gene whose level of expression is reduced in a transgenic insect or insect cell of the invention is preferably the *dhr96* or *EcR* (ecdysone receptor) gene from *D. melanogaster* or a homologue of *dhr96* or *EcR*.

The term "homologue" as used herein with respect to an insect nuclear hormone receptor gene refers to any gene which functions in the same way (i.e. to regulate xenobiotic detoxification) as an insect nuclear hormone receptor gene and/or which exhibits substantial identity to a named insect nuclear hormone receptor gene. Typically a homologue of the invention will be an insect nuclear hormone receptor gene that classifies into the NR1 family of nuclear hormone receptors (Maglich et al. 2001, Genome Biology 2:research 0029.1-0029.7), and especially into the 1H, 1I and 1J sub-families (Cell, 1999, 97:161-163). Preferably a homologue of the invention encodes a protein which exhibits at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity with a nuclear hormone receptor encoded by a named insect nuclear hormone receptor gene. Most preferably a homologue encodes a protein that exhibits at least 96%, 97%, 98% or 99% identity with that encoded by a named insect nuclear hormone receptor gene. In one embodiment a homologue will exhibit at least 80% identity in the DNA binding domain and at least 40% identity in the ligand binding domain in comparison to said domains from a named insect nuclear hormone receptor. In one embodiment the named insect nuclear hormone receptor gene is *dhr96* from *D. melanogaster*.

Homologues include nuclear hormone receptor genes found in the same insect genus and species as the named nuclear hormone receptor gene, as well as nuclear hormone receptor genes from insects of other genera and/or species. Thus the homologue may come from the group of insects consisting of: *Drosophila sp. Bombyx mori, Tribolium castaneum, Aedes aegyptii, Anopheles gambiae, Anopheles albimanus,*

Anopheles stephensi, Ceratitis capitata, Pectinophora gossypiella, Helicoverpa zea, Bactrocera dorsalis, Anastrepha suspense, Musca domestica, Stomoxys calcitrans, Heliothis virescens, Manduca sexta and *Lucilia cuprina.* Prefereably the homologue will come from a known insect pest species, including in particular (but not limited to) *Heliothis virescens* and *Manduca sexta.*

The transgenic insect or transgenic insect cell may be any insect or insect cell which is capable of being transformed. In particular the transgenic insect may be, or the transgenic insect cell may come from, any one of the following insects: *Drosophila sp. Bombyx mori, Tribolium castaneum, Aedes aegyptii, Anopheles gambiae, Anopheles albimanus, Anopheles stephensi, Ceratitis capitata, Pectinophora gossypiella,, Helicoverpa zea, Bactrocera dorsalis, Anastrepha suspense, Musca domestica, Stomoxys calcitrans,* and *Lucilia cuprina.* In addition, a transgenic insect cell of the invention may also be derived from *Spodoptera frugiperda* or *Trichoplusia ni.* In one embodiment it is particularly preferred that the transgenic insect cell is derived from a *D. melanogaster* S2 cell.

Insects from the genera *Drosophila*, in particular *Drosophila melanogaster*, *Drosophila simulans* and *Drosophila virilis* are considered as particularly suitable for use in the invention. In one preferred embodiment, transgenic insects or insect cells of the invention are derived from *D. melanogaster.*

Transgenic insects and insect cells according to this aspect of the invention exhibit a reduced level of expression of an insect nuclear hormone receptor gene in comparison to the level of expression of the nuclear hormone receptor gene in a wild-type non-transgenic insect or insect cell. Where the transgenic insect is *D. melanogaster*, *D. melanogaster* Canton S is particularly suitable as a wild-type non-transgenic insect and where the transgenic insect cell is derived from a *Drosophila* cell line, such as the *Drosophila* S2 cell line, the untransformed cell line may act as a suitable non-transgenic insect cell for the purposes of comparison.

For the avoidance of doubt, a reduced level of expression of a nuclear hormone receptor gene includes any level of expression that is less than the level of expression of the nuclear hormone receptor gene in the non-transgenic insect or insect cell and includes no detectable expression of said nuclear hormone receptor gene in the transgenic insect or insect cell.

The reduced level of expression of the nuclear hormone receptor gene in the transgenic insect or insect cell may be effected through gene disruption at the locus for the nuclear hormone receptor gene in the insect concerned. This may achieved through homologous recombination (see Rong & Golic 2000 Science 288(5473):2013-2018 for methodology relating to homologous recombination in *Drosophila*) or through insertional mutagenesis e.g. transposon insertion.

Alternatively the reduced level of nuclear hormone receptor expression may be effected using an RNA-interference (RNAi) mediated knockdown or knockout of nuclear hormone receptor gene function, via either a transient RNAi approach, or via the generation of a stable transgenic insect line (Kennerdell & Carthew 2000, Nature Biotech. 17:896-898. Where a stable *Drosophila* line is produced, it is preferred that the reduction in nuclear hormone receptor gene expression level is inducible. This may be achieved by employing the GAL4-UAS system (Brand & Perrimon, 1993, Development 118:401-415) as described herein in the Examples.

In one embodiment of the invention where the reduction in the level of nuclear hormone receptor gene expression is mediated through an RNAi approach, an insect or insect cell is transformed with a DNA construct comprising a first DNA sequence encoding a fragment of an insect nuclear hormone receptor gene and a second DNA sequence which is the reverse complement of the first DNA sequence, wherein the first and second DNA sequences are present on the same strand of DNA. Upon transcription a mRNA molecule will be produced that is capable of forming a hairpin or stem-loop structure by virtue of the complementarity of the regions in the mRNA which correspond to the first and second DNA sequences. This double stranded mRNA region acts as a source of short interfering RNA molecules and thus mediates the knockdown or knockout of nuclear hormone receptor gene function, which is manifested in transgenic insects or insect cells as a reduced level of expression of the nuclear hormone receptor gene.

Thus in a further aspect the invention provides a DNA construct comprising a first DNA sequence encoding a fragment of an insect nuclear hormone receptor gene and a second DNA sequence which is the reverse complement of the first DNA sequence, wherein the first and second DNA sequences are present on the same strand of DNA and are operably linked to a promoter region and optionally a terminator region.

It is thought that in order for the level of gene expression to be reduced through an RNAi mediated mechanism, the minimum size of a short interfering RNA is between 21

and 23 nucleotides (Zamore *et al.* 2000 Cell 101:25-33). Accordingly for just a single short interfering RNA to be produced from the transcript of a DNA construct of this aspect of the invention, the minimum length of the first and second DNA sequences in said DNA construct is 23 nucleotides. However, it is preferred that the length of the first and second DNA sequences is considerably longer than 23 nucleotides. Preferably the first and second DNA sequences will be greater than or equal to 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or 1500 nucleotides in length.

In one embodiment a DNA construct of the invention will additionally comprise a region of DNA which acts as a spacer region between the first and second DNA sequences. Such a DNA sequence may be incorporated to facilitate cloning or to facilitate the formation of a double-stranded mRNA structure, such as a hairpin or stem-loop structure, when the DNA sequence of the DNA construct is transcribed.

Sequences that are known to be introns in a gene of the insect that is to be transformed with a DNA construct of the invention are particularly suitable for this purpose.

The first and second DNA sequences that are present in a DNA construct of the invention are operably linked to a promoter region. In general the promoter region will be any promoter region capable of driving expression of the first and second DNA sequences in the insect or insect cell into which a DNA construct of the invention is to be introduced. The promoter region may comprise a constitutive promoter, a tissue- or developmentally-specific promoter, or an inducible promoter. Preferably the promoter region comprises an inducible promoter so that the reduction in the level of expression of the nuclear hormone receptor gene is controllable. Preferably the inducible promoter comprises UAS hsp70 TATA promoter sequences responsive to the GAL4 protein.

DNA constructs of the invention optionally comprise a transcriptional terminator region. Typically, such a transcriptional terminator region will be one that is suitable for the termination transcription in the insect/insect cell into which the DNA construct is to be introduced. Transcriptional terminator regions suitable for this purpose are described in the art.

DNA constructs of the invention are introduced into insect cells to form transgenic insects and insect cells using any suitable method available in the art. In particular, *D. melanogaster* embryos may be transformed using the methodology described by

Spradling & Rubin (1982, Science218:341-347), for example, as described herein in the Examples.

Where the promoter region of a DNA construct of the invention comprises a constitutive promoter region, transformation of the DNA construct into an insect or insect cell will yield a transgenic insect or insect cell of the invention. Thus, a transgenic insect or insect cell of the invention may be produced merely by transforming an appropriate insect or insect cell with a DNA construct as described hereinbefore.

However, where the promoter region comprises an inducible promoter an inducing agent must be applied to (or be present within) the transformed insect or insect cell.

In *Drosophila* this may be achieved through the use of a binary system as described by Brand & Perrimon (1993, Development 118:401-415) and as used herein in the Examples. Essentially a DNA construct of the invention is introduced into a first insect and a second DNA construct, which encodes a protein capable of regulating expression from the inducible promoter in the DNA construct of the invention, is introduced into a second insect. The first and second insects are then genetically crossed to yield progeny, a proportion of which will comprise both the first and second DNA constructs. Where expression of the protein encoded by the second DNA construct is under the control of a constitutive promoter, the population of progeny comprising both the first and second DNA constructs will comprise not only a DNA construct of the invention but also the inducing agent necessary to effect induction from the promoter region in the first DNA construct. Consequently the population of progeny, which comprises both the first and second DNA constructs, consists of transgenic insects of the invention.

Thus in a further aspect of the invention there is provided a method of producing a transgenic insect of the invention, which comprises: (i) transforming a first insect with a first DNA construct as described hereinbefore wherein the promoter region comprises an inducible promoter; (ii) transforming a second insect with a second DNA construct encoding a protein that is capable of inducing expression from the inducible promoter in the first DNA construct; (iii) crossing the transformed insects resulting from steps (i) and (ii) to obtain progeny; wherein a population of the progeny resulting from step (iii) comprises both the first and the second DNA constructs and when the protein encoded in the second DNA construct is expressed, the level of expression of the nuclear hormone

receptor gene is reduced in said population of the progeny relative to the level of expression of the nuclear hormone receptor gene in a non-transgenic insect.

Insects containing the second DNA construct (e.g. insects already transformed with a DNA construct encoding a protein that is capable of inducing expression from the inducible promoter in the first DNA construct are also readily available in the art (see Example 4 hereinafter). Accordingly the invention also provides a method of producing a transgenic insect of the invention, which comprises: (i) transforming a first insect with a first DNA construct as described hereinbefore wherein the promoter region comprises an inducible promoter; (ii) crossing the transformed insect resulting from step (i) with an insect that expresses an inducing protein that is capable of inducing expression from the inducible promoter in the first DNA construct, to obtain progeny; wherein a population of the progeny resulting from step (ii) comprise the first DNA construct and express the inducing protein so that the level of expression of the nuclear hormone receptor gene is reduced in said population of the progeny relative to the level of expression of the nuclear hormone receptor gene in a non-transgenic insect.

In a similar manner, a transgenic insect cell may be made by co-transforming a suitable insect cell with a DNA construct of the invention and a second DNA construct that encodes a protein capable of regulating expression from the inducible promoter in the DNA construct of the invention.

The transgenic insects and transgenic insect cells of the invention are more susceptible to compounds that are subject to xenobiotic detoxification wherein the xenobiotic pathway is regulated by the insect nuclear hormone receptor gene whose expression level has been reduced therein. Thus the transgenic insects and insect cells of the invention are useful in identifying compounds which are potential insecticides and which would not have been identified, or would have only been identified as being weakly active, in a conventional screen using a wild-type and/or non-transgenic insect or insect cell.

Thus in a further aspect of the invention there is provided a method of identifying or verifying the ability of a compound to act as an insecticide, which comprises: (i) placing a transgenic insect or insect cell of the invention in contact with the compound; (ii) assessing the transgenic insect or insect cell that has been placed in contact with the compound in step (i) for any deleterious effect on the insect or insect cell; wherein the

presence of a deleterious effect is indicative that said compound is capable of acting as an insecticide.

A deleterious effect may be manifested as the death of the insect or insect cells, or by slow growth of the insect or insect cell in comparison to insect or insect cells that have not been treated with the compound, or by some other clear phenotypic change in treated insects or insect cells in comparison to untreated insects or insect cells.

The present invention also provides assays for identifying insecticidal compounds that are not susceptible to detoxification via a pathway regulated via an insect nuclear hormone receptor, in particular compounds that are resistant to degradation by the products of insect *cyp* gene or genes that are regulated by an insect nuclear hormone receptor. Furthermore the invention provides a method of reducing the susceptibility of a compound to detoxification by a pathway that is regulated by an insect nuclear hormone receptor.

These assays utilise transgenic insect and insect cells as described hereinbefore as well as cells and/or whole organisms that over-express an insect nuclear hormone receptor gene, or which express an insect nuclear hormone receptor gene in a heterologous manner.

Where these assays involve the use of cells and/or whole organisms that over-express an insect nuclear hormone receptor gene, or which express an insect nuclear hormone receptor gene in a heterologous manner such cells and/or whole organisms may comprise a DNA construct comprising the *dhr96* gene *Drosophila melanogaster*, or a homologue thereof, operably linked to a promoter region and optionally a transcriptional terminator. Such DNA constructs are referred to herein as "expression constructs", and form yet a further aspect of the invention. It is preferred that the nuclear hormone receptor gene will be selected from the group consisting of *dhr96* from *D. melanogaster, EcR* from *D. melanogaster* and homologues thereof derived from the following insects: *Drosophila sp. Bombyx mori, Tribolium castaneum, Aedes aegyptii, Anopheles gambiae, Anopheles albimanus, Anopheles stephensi, Ceratitis capitata, Pectinophora gossypiella, Helicoverpa zea, Bactrocera dorsalis, Anastrepha suspense, Musca domestica, Stomoxys calcitrans, Heliothis virescens, Manduca sexta* and *Lucilia cuprina*. It is particularly preferred that nuclear the hormone receptor gene is *dhr96* or a homologue thereof.

In general the promoter region to which the insect nuclear hormone receptor gene is operably linked will be any promoter region capable of driving expression of the insect

nuclear hormone receptor gene in the host cell into which an expression construct comprising the insect nuclear hormone receptor gene is to be introduced. Thus, if it is desired that the expression construct be introduced into a bacterial cell, the promoter will be operable in that bacterial cell. Similarly if it is desired that the expression construct be introduced into a yeast cell, the promoter will be operable in that yeast cell and the same logic prevails if the construct is to be introduced into an insect cell or a mammalian cell. Accordingly the promoter region may thus be the native promoter that is associated with the individual insect nuclear hormone receptor gene in nature, provided that the promoter is capable of driving expression in the host cell into which the construct is to be introduced. Alternatively the promoter region may be any one of those with which the skilled man is familiar with for mediating expression in the host of choice. Thus, the promoter region may comprise a constitutive promoter, a tissue- or developmentally-specific promoter, or an inducible promoter. In a particularly preferred embodiment, where the baculovirus expression system and *Sf9* or *Sf21* cells are to be employed the promoter region will comprise the polyhedrin promoter from the *Autographa californica* nuclear polyhedrosis virus.

Expression constructs according to this aspect of the invention optionally comprise a transcriptional terminator region. Typically, such a transcriptional terminator region will be one that is suitable for termination transcription in the host cell into which the expression construct is to be introduced. Transcriptional terminator regions suitable for this purpose are described in the art.

The present invention also provides a cell transformed with an expression construct as described hereinbefore. Suitable host cells for transformation with a DNA construct of the invention include bacterial cells (such as *Escherichia coli*), yeast cells (for example, *Saccharomyces cerevisiae*, *Pichia* species, *Schizosaccharomyces pombe*), insect cells (for example, *Drosophila* S2 cells, *Spodoptera frugiperda* Sf9 or Sf21 cells, *Trichoplusia ni* High Five™ cells), and mammalian cells. In a particularly preferred embodiment, an expression construct of the invention is transformed into insect cells, in particular Sf9 cells.

Introduction of the expression construct into a host cell may employ any suitable technique. In eukaryotic cells such techniques include calcium phosphate transfection, DEAE-Dextran, electroporation, particle bombardment, liposome-mediated transfection or transduction using retrovirus, adenovirus or other viruses, such as vaccinia or, for

insect cells, baculovirus. In bacterial cells, suitable techniques may include calcium chloride transformation, electroporation or transfection using bacteriophage. In particularly preferred embodiment, Sf9 cells are transformed using baculovirus, using for example, the protocols supplied with the MaxBac®2.0 –Complete Baculovirus Expression System (Invitrogen).

The expression construct may also be introduced into a whole organism i.e. an insect, and a stable insect line generated. In one embodiment it is preferred that the expression construct is introduced into *Drosophila*, using standard transformation techniques (see for example Spradling & Rubin 1982, Science 218:341-347). Thus transgenic *Drosophila* insects may be produced wherein the level of expression of the insect nuclear hormone receptor gene present in the expression construct is amplified over the level of expression observed in wild-type non-transformed *Drosophila*.

In yet another aspect of the invention a naturally occurring nuclear hormone receptor in a host insect (or insect cell) may be replaced with a homologue from a different insect species. Typically the host will be a model insect species that is preferably not an agronomic pest-species. *Drosophila* species are a suitable host species for use in this aspect of the invention. Preferably the homologue that is used to replace the naturally occurring nuclear hormone receptor will be derived from one of the following insect species: *Bombyx mori, Tribolium castaneum, Aedes aegyptii, Anopheles sp., Ceratitis capitata, Pectinophora gossypiella, Heliothis virescens, Helicoverpa armigera, Helicoverpa zea, Batrocera dorsalis, Anastrepha suspense, Musca domestica, Stomoxys calcitrans, Lucilia cuprona.*

Cells or insects transformed with an expression construct of the invention may be employed in a method of assaying the susceptibility of a compound to detoxification via a pathway regulated by the nuclear hormone receptor gene present in the expression construct. The method comprises: a) culturing in a suitable growth medium a population of cells or an insect transformed with a DNA construct of the invention b) incubating said compound with said population of cells or an insect; and c) determining after said incubation the level of one or more degradation products of said compound in said population of cells or said insect; wherein the presence of a measurable level of one or more degradation products is indicative that said compound is susceptible to degradation by the product of a gene regulated by the nuclear hormone receptor gene present in the expression construct.

The suitable growth medium will be dependent upon the type of transformed cells of the invention. For example, if the transformed cells of the invention are *Saccharomyces cerevisiae* yeast cells, YPD is one such suitable medium. Where the transformed cells are Sf9 or Sf21 cells, Grace's Insect Cell Culture Medium is one such medium. Where the transformed cells are Drosophila S2 cells, suitable culture medium is Schneider's insect medium, optionally supplemented with foetal calf serum. The skilled man will be familiar with alternative media that are suitable for growth of the particular host cells chosen.

Degradation products of the compound being assayed may be measured using any suitable analytical technique. In a preferred embodiment LC-MS is the technique employed to detect and measure metabolites. In order to facilitate the measurement of metabolites, the cells or insects treated with a compound may be lysed and the cell contents released into a suitable buffer. The skilled man will be familiar with suitable methods for cell lysis which are well-documented in the art.

By comparing the effect of a compound on an insect or insect cell under-expressing an insect nuclear hormone receptor gene with the effect of the same compound on an insect or cell over-expressing the same insect nuclear hormone receptor gene (and optionally also comparing the effect of the compound on a wild type and/or non-transformed insect or insect cell), the susceptibility of that compound to detoxification via a pathway regulated via the encoded nuclear hormone receptor may be assessed. Such a test allows better detection of weak insecticidally active compounds that may otherwise not have been identified, since they are susceptible to detoxification by a pathway that is regulated by the insect nuclear hormone receptor. The *in vivo* metabolism of the compound may then be examined using any suitable analytical technique (for example, an LC-MS technique) and solutions proposed for the enhancement of stability of the compound by altering the chemistry of those parts of the molecule that are subject to degradation. The use of transgenic insects, insect and other cells in this way forms yet another aspect of the invention.

Thus a compound that has been identified or verified as being capable of acting as an insecticide using a transgenic insect or insect cell as described in the first aspect of the invention, may also be tested as described above to assess whether it is susceptible to detoxification via a pathway that is regulated via the nuclear hormone receptor gene whose expression level was reduced in the transgenic insect or insect cell against which

the compound has been tested. It will be appreciated by the skilled man that where a compound is tested in this manner, the same nuclear hormone receptor gene will be used in each assay. It will also be appreciated that the assay for the susceptibility to detoxification may be performed initially, and the assay using a transgenic insect or insect cell as described in the first aspect of the invention may be carried out subsequently. Where this is the order in which the assays are carried out, the presence of degradation products in the first assay should correlate with the observation of insecticidal activity in the second assay.

Where it is found that a compound being assayed is susceptible to metabolic detoification, the assay (or assays) may be used in an iterative manner to aid the design of compounds that are less susceptible to detoxification. For example, if a potential insecticide comprises a backbone chemical structure to which one or more sub-groups are attached, it may be found that by substituting one or more of such sub-groups for a different sub-group and re-testing the modified compound, the modified compound may be found to have an improved resistance to detoxification. If this is the case, the process may be repeated either altering the same, or a different, sub-group and then re-testing the compound in the assay(s) of the invention. In this way compounds may be modified to produce insecticides that are resistant to detoxificaion whichever pathway(s) is(are) being regulated via the under- or over-expressed insect nuclear hormone receptor.

In a further aspect of the invention there is provided a method of identifying or verifying the ability of a compound to act as an insecticide, which comprises: a) culturing in a suitable growth medium a population of insect cells transformed with an expression construct of the invention; b) incubating said compound with said population of cells; and c) determining after said incubation the viability of said cells; wherein a lack of viability of said cells is indicative that said compound is capable of acing as an insecticide.

A lack of viability of cells is preferably indicated by cell death. However, the slow growth of cells treated with the compound in comparison to cells that have not been treated with the compound, or some other clear phenotypic change in treated cells in comparison to untreated cells, may also be used as a measure of cell viability.

A still further aspect of the invention is a method of identifying a compound that regulates expression of an insect nuclear hormone receptor gene, which comprises: (i) culturing in a suitable growth medium a population of cells transformed with a DNA construct comprising a reporter gene operably linked to the promoter region of the nuclear

hormone receptor gene; (ii) incubating said compound with a population of cells according to step (i); (iii) determining the level of expression of said reporter gene in cells that have been incubated with said compound; and (iv) determining the level of expression of said reporter gene in a population of cells according to step (i); wherein, a difference in the levels of reporter gene expression determined at (iii) and (iv) is indicative that said compound is capable of acting as a regulator of expression of said nuclear hormone receptor gene.

DNA constructs for use in this aspect of the invention are referred to herein as "reporter gene constructs" and will comprise any suitable reporter gene available in the art, for example the reporter gene may be a gene encoding : β-galactosidase, luciferase, or a fluorescent protein, such as for example green fluorescent protein. The reporter gene will be operably linked to the promoter region of an insect nuclear hormone receptor gene. Preferably the promoter region will comprise the promoter of the *dhr96* gene from *D. melanogaster*. The promoter regions for use in this aspect of the invention may be isolated and cloned using standard molecular biological techniques.

A yet further aspect of the invention comprises a reporter gene construct wherein the reporter gene is placed under the control of a DHR96 responsive element (or other insect nuclear hormone receptor responsive element), for example a promoter region (e.g. a *cyp* gene promoter region) that is regulated by DHR96 (or other insect nuclear hormone receptor). In one embodiment such a reporter construct is introduced into a transgenic insect or transgenic insect cell in which DHR96 (or insect nuclear hormone receptor homologue) is over-expressed or heterologously expressed. Such a transgenic insect or insect cell is then used to identify compounds that interact with the DHR96 (or other insect nuclear hormone receptor) ligand binding domain thus causing DHR96 (or other insect nuclear hormone receptor) to interact with the response element driving the reporter gene.

A reporter gene construct for use in this aspect of the invention may be transformed into *Drosophila* S2 cells using any suitable method available in the art. Where the reporter gene is luciferase the level of reporter gene expression may be assayed using the Luciferase Assay System from Promega.

Compounds that are identified according to the methods of the invention that utilise reporter gene constructs may be useful as additives to existing insecticides that are

subject to detoxification via a pathway that is regulated by DHR96, or they may find utility as insecticides in their own right.

For the avoidance of doubt, where a compound is to be "identified" as having the ability to act as an insecticide or to act as a regulator of the expression of an insect nuclear hormone receptor gene, that compound has not previously been shown to act as an insecticide or to act as a regulator of expression of an insect nuclear hormone receptor gene. Thus any compound that has previously been shown to act in such a way is excluded from the scope of any method of "identifying" a compound.

Where a compound is to be "verified" as having the ability to act as an insecticide, the compound will already have been shown to have insecticidal activity (although such activity may be weak) through some other means.

In yet a further aspect of the invention there is provided an antibody against an insect nuclear hormone receptor, in particular against DHR96 of *D. melanogaster*. Such an antibody may polyclonal or monoclonal and produced using standard immunological techniques. Antibodies of the invention are useful in verifying whether a particular insect nuclear hormone receptor is expressed in a transgenic insect or insect cell of the invention, and also in quantifying the level of such expression.

Various aspects and embodiments of the present invention will now be illustrated in more detail by way of example. It will be appreciated that modification of detail may be made with out departing from the scope of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Shows a schematic representation of DNA construct "pBUGIR_dhr96"

EXAMPLES

Example 1 Construction of vectors for use in the production of insects wherein the level of expression of an insect nuclear hormone receptor gene is reduced relative to wild type insects

Constructs were generated that encode inverted repeats of *dhr96* gene fragments, which on expression fold to form an RNA duplex or as referred to herein a hairpin (hp) RNA molecule, which is capable of mediating a RNA interference (RNAi) effect. The constructs generated also include a spacer sequence, which serves to facilitate cloning,

between the inverted repeats. The expression of the inverted repeats is under control of an inducible promoter system.

The constructs were generated using site-specific recombinase mediated cloning (1.1). To provide *Drosophila* DNA template for the cloning, genomic DNA from a wild-type lab strain, *Canton-S* (Bloomington stock centre, stock number 1; http:/flystocks.bio.Indiana.edu, Drosophila Stock Center, Department of Biology, Indiana University, 1001 E. 3rd St., Bloomington, IN 47405-3700, USA) was isolated using standard molecular biology techniques (Sambrook, *et al.* 1989, Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory, New York; Bender *et al.* 1983 J. Mol. Biol. 168: 17-33). This was then used as the DNA template for PCR amplification in various steps as described below. The mRNA sequence of *dhr96* (EMBL database, accession No: DM36792) that was used for the design of hairpin constructs is shown below as SEQ ID NO 1. The *dhr96* coding sequence is marked with the start codon and termination codon in bold and underlined. Bases 58-661 of this sequence (shown in italics and in capital letters) were used to generate the hairpin molecule. This cloned region fell within a single exon of the gene.

SEQ ID NO 1:

gttcattaaa atatgtggtg ataacgcgag ctgccgaatc tgcgtgcaat tcgtgcgt*TT* 60
GACGTGGGTA CTAACTGCTA TGCTGTCGCG CGGACAGTTG TTCTGATACG CAGAGTTCCT 120
GCCTCACCAC ACACGACCAC CTCCATTAAA ACCAGCCACC CCCCCCAGCG CCTCCTCCAC 180
CGACAGCAGC TGCTCCACCG CACCACCAGG AGAGGGGCAA TTAAAAAATC AATCAGAGGG 240
*CCCTAATTGA AAGCTGCCAC CGTCGAA**ATG** TCGCCGCCGA AGAACTGCGC GGTGTGCGGG* 300
GACAAGGCTC TGGGCTACAA CTTCAATGCG GTCACCTGCG AGAGCTGCAA GGCGTTCTTC 360
CGACGGAACG CGCTGGCCAA GAAGCAGTTC ACCTGCCCCT TCAACCAAAA CTGCGACATC 420
ACTGTGGTCA CTCGACGCTT CTGCCAGAAA TGCCGCCTGC GCAAGTGCCT GGATATCGGG 480
ATGAAGAGTG AAAACATTAT GTCCGAGGAG GACAAGCTGA TCAAGCGGCG CAAGATCGAG 540
ACCAACCGGG CCAAGCGACG CCTCATGGAG AACGGCACGG ATGCGTGCGA CGCCGATGGC 600
GGCGAGGAAA GGGATCACAA AGCGCCGGCG GATAGCAGCA GCAGCAACCT TGACCACTAC 660
Tcggggtcac aggactcgca gagctgcggc tcggcggaca gcggggccaa tgggtgctcc 720
ggcagacagg ccagttcgcc gggcacacag gtcaatccgc ttcagatgac ggccgagaag 780
atagtcgacc agatcgtatc cgacccggat cgagcctcgc aggccatcaa ccggttgatg 840
cgcacgcaga aagaggctat atcggtgatg gagaaggtaa tcagctcaca aaaggacgcc 900
ttaaggctgg tgtcgcattt gatcgactat ccaggcgacg cactcaagat catttcaaag 960
tttatgaact cgcccttaa cgcgctgaca gtattcacca aattcatgag ctcacccacg 1020

gacggcgttg aaattatctc aaagatagtt gattcgcccg cggacgtggt ggagttcatg 1080
cagaacttga tgcactcgcc agaggacgcc atcgatataa tgaacaagtt catgaatacc 1140
ccagcggagg cgctgcgcat tcttaaccga atcctaagcg gcggaggagc gaacgcagcc 1200
cagcagacag cagaccgcaa gccattgctg gacaaggagc cggcggtgaa gcctgcagcg 1260
ccagcggagc gagctgatac tgtcattcaa agcatgctgg gcaacagtcc gccaatttcg 1320
ccacatgatg ctgccgtgga tctgcagtac cactcgcccg gtgtcgggga gcagcccagt 1380
acatcgagta gccacccctt gccttacata gccaactcgc cggacttcga tctgaagacc 1440
ttcatgcaga ccaactacaa cgacgagccc agtctggaca gtgattttag cattaactca 1500
atcgaatcgg tgctatccga ggtgatccgc attgagtacc aggccttcaa tagcatacaa 1560
caagcggcat cgcgcgtaaa ggaggagatg tcctacggca ctcagtctac gtacggtgga 1620
tgcaattcgg ctgcaaacaa tagccagccg cacctgcagc aacccatctg cgccccatcc 1680
acccagcagt tggatcgcga gctaaacgag gcggagcaaa tgaagctgcg ggagctgcga 1740
ctggccagcg aggctcttta tgatcccgtg gacgaggacc tcagcgccct gatgatgggc 1800
gatgatcgca ttaagcccga cgacactcgc cacaacccaa agctattgca gctgatcaat 1860
ctgacggcgg tggccatcaa gcggcttatc aaaatggcca agaagattac agcattccgt 1920
gacatgtgcc aggaggacca ggtggcccta ctcaaaggtg gctgcacaga aatgatgata 1980
atgcgctccg taatgattta cgacgacgat cgcgccgcct ggaaggtacc ccataccaaa 2040
gagaacatgg gcaacatacg cactgacctg ctcaagtttg ccgaaggcaa tatctacgag 2100
gagcaccaaa agttcatcac aacgtttgac gagaagtggc gcatggacga gaacataatc 2160
ctgatcatgt gtgccattgt ccttttttacc tcggctcgat cgcgagtgat acacaaagac 2220
gtgattagat tggaacagaa ttcctactat tatcttctgc gaagatatct ggagagtgtt 2280
tattctggct gtgaggcgag aaacgcgttt atcaagctaa tccaaaagat ttcagatgtg 2340
gagcgtctga acaagttcat aattaatgtc tatttgaatg ttaacccatc ccaggtggag 2400
cccttgctgc gtgaaatatt cgatttgaaa aatcac**tag**a caaccgatgc gtgtcgggca 2460
tttaatgcct atgttgatgc ccaatgatga atggtcaaca agctgtagtt gttgttgttg 2520
ttgatgtctg ttttatcttg tcgcttgtaa tgttagattt taatcgaatg tgattgttag 2580
atttgcatat actgcataga ttttatattt ctacatcaaa gagagcatat ttaggatacc 2640
aagtgcaaag caacacaatc tatatgtaat gtacaccgtt tacctagttt caaataaact 2700
agacgataat gcaataacta acttggaagc gtgggttctg tgcaaaaagg aaaaaagaca 2760
aaaaaaataa actgactttg agaaccagtg gtaaacc 2797

1.1 Site-specific recombinase mediated cloning

The strategy employed in the production of hairpin constructs utilises site-specific phage recombinase mediated cloning (Gateway technology, Invitrogen). The pB-UGIR w+ vector (Genbank AY196824) was used as it has been engineered to contain recombinase target sites that allow recombination of inverted repeats into the vector.

The insert sequence for *dhr96* (bases 58-661 of SEQ ID NO 1) was PCR amplified using primers dhr96-F (SEQ ID NO 4:5' tattgcggatccttgacgtgggtactaactgctatg 3') and

dhr96-R (SEQ ID NO 5: 5' agtccggaattcagtagtggtcaaggttgctgct 3') containing the appropriate recombinase target site sequences which facilitate recombinase-mediated cloning into ENTRY vectors (Invitrogen). Then, in a single step, these ENTRY-derived plasmids were mixed with the pB-UGIR w+ vector, and the desired constructs were generated via recombinase-mediated cloning following the manufacturer's protocols. Using the pB-UGIR w+ vector, the *dhr96* sequence inserts were cloned in the tail-to-tail orientation (i.e. the first insert was cloned 5' to 3' with the second being cloned 3' to 5', see Figure 1).

Example 2 Transformation of *D. melanogaster* and generation of transgenic flies

Dropsophila melanogaster w^{1118} embryos (Bloomington stock centre, stock No. 6326) were transformed according to Spradling,A.C. and Rubin,G.M (1982, Science 218:341-347). Briefly, the DNA plasmids for transformation were prepared using a Qiagen Midi kit. Each transformation construct was then co-precipitated and resuspended into injection buffer (5 mM KCl, 0.1 mM NaH_2PO_4, pH 6.8) at a concentration of 0.5-1 µg/µl, together with the piggyBac helper plasmid (pBlu-uTp, Genbank AY196821) at a concentration of 0.1 µg/µl. Embryos were injected prior to pole cell formation; thus 0-30 min old w^{1118} embryos were collected, dechorionated and dessicated, and then lined up on glass slides covered with double-sided sticky tape to hold the embryos in place. The embryos were covered with a minimal amount of halocarbon oil, and microinjected posteriorly with the transformation construct and helper plasmid mix. The injected embryos were then incubated at 25°C, and any hatching larvae were recovered and transferred to standard diet. Adults emerging from these larvae were then crossed to w^{1118} adults, and stable transformants were selected from their progeny (expressing the *mini-w+* marker for transformation). Several independent transgenic flies per injected construct were isolated, and stocks of these transgenic flies were established. Transgenics were also confirmed by PCR amplification of sequences specific to the transformation plasmids.

Example 3 Growth and Maintenance of *D. melanogaster* flies

Drosophila melanogaster flies were cultured according to standard methods (as described in http://flystocks.bio.indiana.edu/culturing.htm). The flies were fed on a diet that was made as follows: 100g agar, 350g yeast, 300g treacle, 150g sucrose, 300g dextrose, 150g maizemeal, 100g wheatgerm, 200g soya flour were mixed in a total of 10 litres of water,

and the mixture heated to ensure thorough mixing and melting of the agar. After allowing the diet to cool to below 60°C, 10 g nipagen (4-hydroxybenzoic acid methylester) in 10 ml ethanol and 50 ml of proprionic acid was mixed into the diet. The diet was dispensed into culture bottles or vials, and allowed to set before use. The flies were routinely cultured in this diet, and maintained in a 70-75% humidified 18°C or 25°C incubator.

Example 4 Induction of expression of the *dhr96* hairpin RNA molecule using a binary component system

The transgenic flies generated in Example 2 comprise a *dhr96*-specific hairpin RNA molecule, and the expression of this hairpin molecule is under the control of an inducible promoter. Induction of hairpin expression mediates the degradation of the corresponding endogenous mRNAs through an RNA interference (RNAi) mechanism. As expression of the hairpin molecules is under control of an inducible promoter, an inducing agent is provided to effect the RNAi in these flies. This inducing agent is provided from another transgenic line by means of a genetic cross that brings together the inducing agent and the inducible transgene (i.e. in this case the hairpin molecule) into the same individual flies.

In this type of binary component system, the yeast GAL4 protein acts as a transcriptional regulator and thus as an inducing agent; it binds to UAS promoter sequences and activates the expression of any sequences encoded downstream of this promoter, thus rendering the expression of such sequences inducible by the GAL4 protein (Brand,A.H & Perrimon,N 1993, Development 118:401-415).

This binary system is applied by a genetic cross, between (a) a transgenic fly strain that encodes for the transcription factor (GAL4), and (b) a transgenic fly line that contains the UAS promoter encoded upstream of the cloned *dhr96* inverted repeat sequences (generated as described in Example 2). A proportion of the progeny from such a cross will express both the GAL4 protein as well as the hairpin molecule (via GAL4 regulation), and express double-stranded (ds) RNA corresponding to partial *dhr96* sequences encoded by the hairpin. These dsRNA molecules then mediate degradation of the endogenous mRNA transcripts for *dhr96*, via an RNAi mechanism. The result is that the amount of endogenous mRNA transcripts for *dhr96* is depleted. This effects a reduced level of expression of the *dhr96* gene and the product thereof, and a knockdown or knockout of *dhr96* gene function is thus observed.

The GAL4-expressing transgenic fly lines used herein was obtained from the Bloomington stock centre (*supra*) and are described by the genotype: *y[1] w[*]*; *P{w[+mC]=Act5C-GAL4}25FO1/CyO, y[+]* (Bloomington stock number 4414); *w[*]; P{w[+mW.hs]=GAL4-arm.S}11* (Bloomington stock number 1560). The transgenic fly line comprising *dhr96* inverted repeat region was crossed with the GAL-4-expressing transgenic fly lines.

The resulting F1 progeny were selected for those that carried both the GAL4 and the *dhr96_hairpin* transgene. The flies from some of these crosses had visible phenotypes which were attributed to the knockdown of *dhr96* expression.

At least three independent transgenic lines encoding *dhr96* sequence hairpin molecules were used in independent genetic crosses to ensure that the effects seen were not due to position effects of the transgene insertion site.

Example 5 Generation of anti-DHR96 polyclonal antibodies

Antibodies were raised to peptides from the DHR96 protein. These peptides were selected (a) to maximise the probability that antibodies generated against them would be specific to the protein from which they derived, and (b) from regions predicted to have good antigenicity (using Windows 32 Protean 5.03 software, DNASTAR Inc.). The nucleotide sequences encoding two peptide fragments from DHR96 (DHR96_peptide 1 SEQ ID NO 2, DHR96_peptide2 SEQ ID NO 3) were cloned and expressed, using standard molecular biology techniques (Sambrook, *et al.* 1989, Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory, New York). These peptides were tagged at the 5' end with maltose-binding protein (MBP) to facilitate later purification steps.

From SWISSPROT protein sequence for DHR96 (Q24143):

DHR96_peptide1 (SEQ ID NO 2; amino acids 74 – 221 from SWISSPROT accession No Q24143):

SENIMSEEDKLIKRRKIETNRAKRRLMENGTDACDADGGEERDHKAPADSSSSNL
DHYSGSQDSQSCGSADSGANGCSGRQASSPGTQVNPLQMTAEKIVDQIVSDPDR
ASQAINRLMRTQKEAISVMEKVISSQKDALRLVSHLIDY

DHR96_peptide2 (SEQ ID NO 3; amino acids 260 – 513 from SWISSPROT accession No Q24143):

TDGVEIISKIVDSPADVVEFMQNLMHSPEDAIDIMNKFMNTPAEALRILNRILSGG
GANAAQQTADRKPLLDKEPAVKPAAPAERADTVIQSMLGNSPPISPHDAAVDLQ
YHSPGVGEQPSTSSSHPLPYIANSPDFDLKTFMQTNYNDEPSLDSDFSINSIESVLSE
VIRIEYQAFNSIQQAASRVKEEMSYGTQSTYGGCNSAANNSQPHLQQPICAPSTQ
QLDRELNEAEQMKLRELRLASEALYDPVDEDLSALMMGDD

The DHR96 fragment-MBP-fusion peptides were expressed and purified. New Zealand white rabbits were immunised with these DHR96 peptide immunogens. The immunoglobulin fractions were purified by affinity chromatography on Protein A-Sepharose. Additionally, anti-MBP antibodies were removed from the immunoglobulin fractions by affinity chromatography on MBP-Sepharose. All of the antibodies were assessed for reactivity towards their immunising peptides by immunoblotting against peptide-MBP fusions. A control of MBP only was included in the immunoblot. Non-specific binding was checked for using pre-immune sera from the rabbits.

Polyclonal antibodies against *Drosophila melanogaster* DHR96 have thus been generated.

CLAIMS

1. A transgenic insect or insect cell, wherein the level of expression of an insect nuclear hormone receptor gene has been reduced relative to the level of expression of said insect nuclear hormone receptor gene in a non-transgenic insect or insect cell,

2. A transgenic insect or insect cell according to claim 1 wherein the insect nuclear hormone receptor gene is selected from the group consisting of *dhr96* and *EcR* genes from *D. melanogaster* and homologues thereof.

3. A transgenic insect or insect cell according to claim 1 or claim 2 wherein the homologue is from an insect selected from the group consisting of *Drosophila sp.*, *Bombyx mori, Tribolium castaneum, Aedes aegyptii, Anopheles gambiae, Anopheles albimanus, Anopheles stephensi, Ceratitis capitata, Pectinophora gossypiella, Helicoverpa zea, Bactrocera dorsalis, Anastrepha suspense, Musca domestica, Stomoxys calcitrans,* and *Lucilia cuprina.*

4. A transgenic insect or insect cell according to claim 1 or claim 2 wherein the insect nuclear hormone receptor gene is *dhr96* or *EcR*.

5. A transgenic insect or insect cell according to any one of the preceding claims wherein the transgenic insect or insect cell is selected from the group of insects consisting of: *Drosophila sp., Bombyx mori, Tribolium castaneum, Aedes aegyptii, Anopheles gambiae, Anopheles albimanus, Anopheles stephensi, Ceratitis capitata, Pectinophora gossypiella, Helicoverpa zea, Bactrocera dorsalis, Anastrepha suspense, Musca domestica, Stomoxys calcitrans,* and *Lucilia cuprina*

6. A transgenic insect or insect cell according to claim 5 which is or comes from a species of *Drosophila*.

7. A transgenic insect or insect cell according to claim 6 which is or comes from *D. melanogaster*.

8. A transgenic insect or insect cell according to any one of the preceding claims wherein the level of expression of the insect nuclear hormone receptor gene has been reduced through RNAi.

9. A transgenic insect or insect cell according to any one of claims 1 to 7 wherein the level of expression of the insect nuclear hormone receptor gene has been reduced through insertional mutagenesis.

10. A transgenic insect or insect cell according to any one of claims 1-7 wherein the level of expression of the insect nuclear hormone receptor gene has been reduced through homologous recombination.

11. A DNA construct comprising a first DNA sequence encoding a fragment of an insect nuclear hormone receptor gene and a second DNA sequence which is the reverse complement of the first DNA sequence, wherein the first and second DNA sequences are present on the same strand of DNA and are operably linked to a promoter region and optionally a terminator region.

12. A DNA construct according to claim 11 wherein the first and second DNA sequences are separated by a third DNA sequence which acts a spacer sequence between the first and second DNA sequences.

13. A DNA construct according to claim 10 or claim 11 wherein the insect nuclear hormone receptor gene is selected from the group consisting of *dhr96* and *EcR* genes from *D. melanogaster* and homologues thereof.

14. A DNA construct according to claim 13 wherein the insect nuclear hormone receptor gene is *dhr96 or EcR*.

15. A DNA construct according to claim 14 which comprises bases 58-661 of SEQ ID NO 1, or the reverse complement thereof.

16. A method of producing a transgenic insect as defined in any one of claims 1-8, which comprises:

(i) transforming a first insect with a first DNA construct according to any one of claims 11 to 15 wherein the promoter region comprises an inducible promoter;

(ii) transforming a second insect with a second DNA construct encoding a protein that is capable of inducing expression from the inducible promoter in the first DNA construct;

(iii) crossing the transformed insects resulting from steps (i) and (ii) to obtain progeny;

wherein a population of the progeny resulting from step (iii) comprises both the first and the second DNA constructs and when the protein encoded in the second DNA construct is expressed, the level of expression of the insect nuclear hormone receptor gene is reduced in said population of the progeny relative to the level of expression of the insect nuclear hormone receptor gene in a non-transgenic insect.

17. A method of producing a transgenic as defined in any one of claims 1-8, which comprises:

(i) transforming a first insect with a first DNA construct according to any one of claims 11 to 15 wherein the promoter region comprises an inducible promoter; and,

(ii) crossing the transformed insect resulting from step (i) with an insect that expresses an inducing protein that is capable of inducing expression from the inducible promoter in the first DNA construct, to obtain progeny;

wherein a population of the progeny resulting from step (ii) comprise the first DNA construct and express the inducing protein so that the level of expression of the insect nuclear hormone receptor gene is reduced in said population of the progeny relative to the level of expression of the insect nuclear hormone receptor gene in a non-transgenic insect.

18. A method of identifying or verifying the ability of a compound to act as an insecticide, which comprises:

(i) placing a transgenic insect or insect cell according to any one claims 1 to 8 in contact with the compound

(ii) assessing the transgenic insect or insect cell that has been placed in contact with the compound in step (i) for any deleterious effect on the insect or insect cell;

wherein the presence of a deleterious effect is indicative that said compound is capable of acting as an insecticide.

19. A method of identifying a compound that regulates expression of an insect nuclear hormone receptor gene, which comprises:

(i) culturing in a suitable growth medium a population of cells transformed with a DNA construct comprising a reporter gene operably linked to the promoter region of the insect nuclear hormone receptor gene

(ii) incubating said compound with a population of cells according to step (i);

(iii) determining the level of expression of said reporter gene in cells that have been incubated with said compound; and

(iv) determining the level of expression of said reporter gene in a population of cells according to step (i);

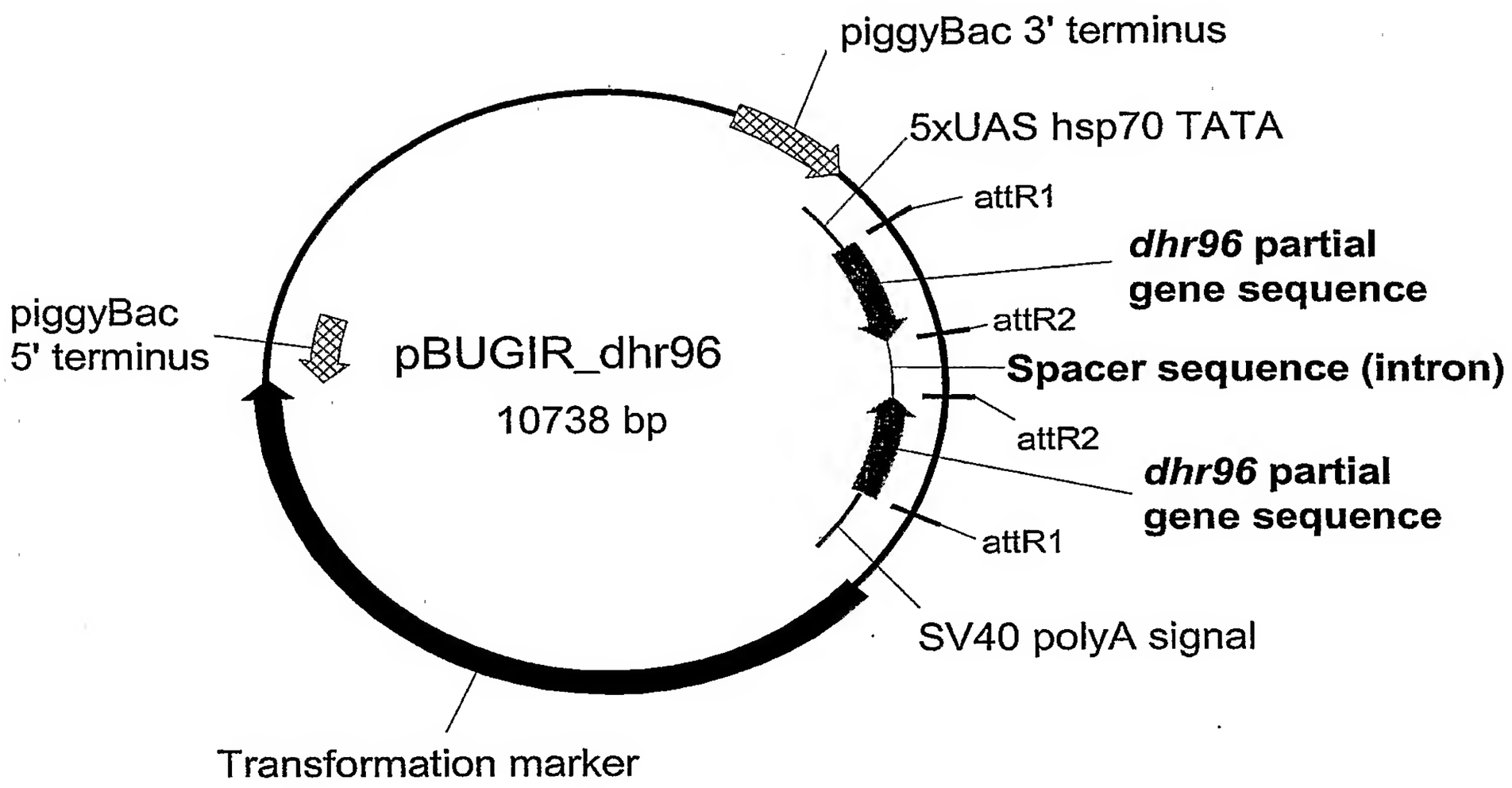
wherein, a difference in the levels of reporter gene expression determined at (iii) and (iv) is indicative that said compound is capable of acting as a regulator of expression of said insect nuclear hormone receptor gene.

20. A transgenic insect or insect cell of a first insect species comprising a nuclear hormone receptor gene from a second insect species.

ABSTRACT

The present invention relates to methods of screening for potential insecticidal agents using genes encoding proteins that regulate xenobiotic detoxification in insects. In particular the invention relates to methods of using insect nuclear hormone receptor genes and/or their promoters. Such methods employ transgenic cells and/or whole organisms in which one or more nuclear hormone receptor gene is deleted or under-expressed.

FIGURE 1


piggyBac 3' terminus
5xUAS hsp70 TATA
attR1
dhr96 partial gene sequence
attR2
Spacer sequence (intron)
attR2
dhr96 partial gene sequence
attR1
SV40 polyA signal
piggyBac 5' terminus
pBUGIR_dhr96
10738 bp
Transformation marker